Behavioral and Neurochemical Effects of 5-{4-[4-(5-Cyano-3-indolyl)-butyl]-butyl]-1-piperazinyl}-benzofuran-2-carboxamide (EMD 68843): A Combined Selective Inhibitor of Serotonin Reuptake and 5-Hydroxytryptamine$_{1A}$ Receptor Partial Agonist

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ABSTRACT

5-{4-[4-(5-Cyano-3-indolyl)-butyl]-butyl]-1-piperazinyl}-benzofuran-2-carboxamide (EMD 68843; vilazodone) is a novel compound with combined high affinity and selectivity for the 5-hydroxytryptamine (5-HT) transporter and 5-HT$_{1A}$ receptors. EMD 68843 was tested as a prototype compound, which benefits from dual pharmacological effects that could increase extracellular 5-HT to levels higher than those produced by conventional selective serotonin reuptake inhibitors (SSRIs). In Sf9 cells, EMD 68843 increased guanosine 5'P/[H11032]-binding to 69% of the magnitude of the full 5-HT$_{1A}$ receptor agonist 8-OH-DPAT, 8-hydroxy-2-(di-propylamino) tetralin; HPv, ventral hippocampus; FC, frontal cortex; FST, forced swimming test; [35S]GTP$_Y/S$, guanosine 5'P/[35S]thiotriphosphate; aCSF, artificial cerebrospinal fluid; DMSO, dimethyl sulfoxide; ANOVA, analysis of variance; PLSD, protected least significant difference; 8-OH-PIPAT, R-(1)-trans-8-hydroxy-2-[N-n-propyl-N-(39-iodo-29-propenyl)] aminotetrailin (8-OH-DA PAT), EMD 68843-induced increases in extracellular 5-HT were greatly reduced in the HPv but to a lesser extent in the FC. In behavioral studies, EMD 68843 produced antidepressant-like effects in the forced swimming test in both rats and mice but only within a narrow dosage range. Like fluoxetine, EMD 68843 did not produce the symptoms of the 5-HT behavioral syndrome. The results show that EMD 68843 augments extracellular 5-HT levels in forebrain regions to a greater extent than fluoxetine. At higher doses, however, weak efficacy of EMD 68843 at postsynaptic 5-HT$_{1A}$ receptors may inhibit the expression of rodent antidepressant-like behaviors.

Selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed class of antidepressant drugs because they are clinically effective, elicit fewer aversive side effects, and have an increased safety margin compared with other antidepressants. SSRIs given acutely block the serotonin transporter and increase extracellular levels of 5-HT in forebrain regions, including the cortex, hippocampus, and striatum (Kreiss and Lucki, 1995; Invernizzi et al., 1996; Romero et al., 1996; Hjorth et al., 2000). The ability of SSRIs to increase serotonergic neurotransmission is believed to be an important component of their clinical antidepressant activity...
EMD 68843 exhibits high affinity for the 5-HT1A receptor (Kreiss and Lucki, 1995; Invernizzi et al., 1996). Unlike the prototypic SSRI fluoxetine, however, which is about 300-fold more selective at inhibiting 5-HT transporters and 5-HT1A receptors, with SSRIs has been shown to enhance the increase of extracellular levels of 5-HT in preclinical studies (Romero et al., 1996) and produce a more rapid onset of clinical antidepressant effects (Blier and Bergeron, 1997; Perez et al., 1997), although not all clinical trials with this drug combination have been positive (Berman et al., 1997).

Serotonergic projections throughout the forebrain arise primarily from two main cell body groups, the dorsal raphe (DR) and median raphe (MR) nuclei (Kosofsky and Molliver, 1987). Regions innervated by the DR (e.g., striatum and frontal cortex) have been shown to be more responsive to regulation by the 5-HT1A autoreceptor than regions innervated by the MR (e.g., dorsal and ventral hippocampus) (Sinton and Fallon, 1988; Hjorth and Sharp, 1991; Malagie et al., 1996; Invernizzi et al., 1997; Romero and Artigas, 1997; Knobelman et al., 2001).

The present studies examined in vivo pharmacological responses evoked by the novel compound EMD 68843 (also known as SB659746-A or vilazodone) because of its dual activity at 5-HT transporters and 5-HT1A receptors. Like SSRIs, EMD 68843 is a potent inhibitor of the reuptake of 5-HT with an IC50 value of 0.2 nM (Bartoszyk et al., 1997), which is about 300-fold more selective at inhibiting 5-HT than nor epinephrine reuptake (C. Seyfried, Merck, unpublished data). Unlike the prototypic SSRI fluoxetine, however, EMD 68843 exhibits high affinity for the 5-HT1A receptor (IC50 = 0.5 nM) (Bartoszyk et al., 1997) and negligible affinity for other 5-HT receptors (5-HT1D, 5-HT2A, and 5-HT2C receptors; C. Seyfried, Merck, unpublished data). Although its efficacy for activating 5-HT1A receptors was not previously determined, in vitro assays demonstrated a decrease in 5-HT release, indicating activation of presynaptic 5-HT1A receptors (Merk, unpublished data). Initial behavioral studies showed that EMD 68843 (55 mg/kg, p.o.) inhibited ultrasonic vocalizations in rats, a behavioral model for anxiolytic effects produced by activation of presynaptic 5-HT1A receptors. The absence of effects on core body temperature of rats was suggested to indicate that EMD 68843 lacked intrinsic activity at postsynaptic 5-HT1A receptors (Bartoszyk et al., 1997). The goal of the present study was to examine the effects of EMD 68843 on extracellular 5-HT levels as a prototype compound expected to demonstrate mixed activity at inhibiting 5-HT reuptake and regulating 5-HT1A receptors. The effects of EMD 68843 were compared with the SSRI fluoxetine on extracellular 5-HT levels in the ventral hippocampus (HPv) and the frontal cortex (FC) after acute, systemic administration using in vivo microdialysis. Additional studies were done to characterize the effects of EMD 68843 on responses mediated by 5-HT1A receptors. For example, the effects of EMD 68843 were examined in the forced swimming test (FST), a behavioral test in rats and mice used to predict antidepressant potential (Borsini and Meli, 1988; Lucki, 1997). Also, the efficacy of EMD 68843 to activate postsynaptic 5-HT1A receptors was evaluated in vitro by examining its ability to promote binding of [35S]GTPyS to G proteins expressed in Sf9 cells efficacy (Butkerait et al., 1995; Barr and Manning, 1997) and in vivo by observing the appearance of the 5-HT behavioral syndrome (Lucki, 1992).

**Materials and Methods**

**Animals.** Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 175 to 200 g at the start of the experiment were housed two per cage and maintained under conditions of constant temperature (22°C) on a 12:12-h light/dark cycle (7:00 AM and 7:00 PM off) with free access to food and water. Animal procedures were conducted in accordance with the guidelines published in the National Institutes of Health Guide for Care and Use of Laboratory Animals, and all protocols were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

After an acclimation period of approximately 1 week, rats were implanted with a stainless-steel-guide cannula directed at either the ventral hippocampus or the frontal cortex using the following coordinates (Paxinos and Watson, 1986) from bregma, midline, and dura using a Kopf stereotactic instrument: anterior-posterior −5.6 mm, mediolateral 4.7 mm, and dorsal-ventral −4.4 mm (HPv), and anterior-posterior +3.2 mm, mediolateral 0.7 mm, and dorsal-ventral −2.5 mm (FC). Cannulae were secured to the skull with cranioplastic cement and skull screws. Rats were housed individually for approximately 1 week following surgery and handled on at least 4 days prior to microdialysis experiments. The day before an experiment, microdialysis probes were lowered into the HPv or the FC, and rats were placed in a cylindrical Plexiglas container lined with bedding material and attached to a liquid swivel by a spring tether. The probes were continuously perfused with an artificial cerebrospinal fluid (aCSF) solution (147 mM NaCl, 1.7 mM CaCl2, 0.9 mM MgCl2, and 4 mM KCl) at a flow rate of 0.8 μl/min. Baseline sample collection began the following morning. Rats received an injection of fluoxetine, EMD 68843, or vehicle (2 ml/kg, i.p.) after 2 h of baseline sampling. Extracellular levels of 5-HT in response to drug injection were measured for 3 h after injection. At the end of the experiment, the probe location was marked by infusion of green food coloring through the dialysis probe. Brains were removed and subsequently sectioned for histological verification of correct probe placement.

**Microdialysis Probe Construction.** Vertical, concentric microdialysis probes were constructed as previously described (Kreiss and Lucki, 1997). A plastic screw cap secured the probe into the guide cannula on the day of the experiment. In vitro recovery rates for dialysis probes were determined by placing them in an Eppendorf tube filled with aCSF. The aCSF was continually perfused through the probe at a rate of 0.8 μl/min. After a known concentration of 5-HT standard was added to the bath, the concentration of 5-HT in the perfusate was compared with the amount in the bath. The reported values for 5-HT in dialysate samples are corrected for recovery for archival purposes. The mean recovery rate was 24 ± 1.2%.

**Dialysate Analysis.** The amount of 5-HT in dialysates was determined with high-pressure liquid chromatography with electrochemical detection. Briefly, 12-μl samples were injected directly onto...
high-pressure liquid chromatography, which consisted of a PM80 solvent delivery system (Bioanalytical Systems, West Lafayette, IN), a 10-μl sample loop, and a Sepstik microbore column (ODS 3 μm; 100 × 1 mm; Bioanalytical Systems). The mobile phase consisted of 32 mM NaH₂PO₄, 0.67 μM EDTA, 0.43 mM octyl sulfate, and 19% methanol adjusted to a pH of 4.3. The flow rate through the system was 110 μl/min, and the detector was set at a potential of +0.60 V relative to a silver/AgCl reference electrode. Standard concentrations of 5-HT were prepared prior to injection of tissue samples. Dialysate concentrations of 5-HT were determined using a linear regression analysis of the peak heights obtained from a range of standard concentrations.

Data Analysis. Microdialysis data are reported as a percentage of the control baseline values (determined from the mean of the final three samples prior to drug injection). The transformed value (percentage of baseline) was used as the dependent variable for assessment of within-group effects. Values for the mean and the nine samples collected immediately after drug injection were included in the analyses. Between-group analyses of dose differences or treatment differences (EMD versus fluoxetine) were conducted using two-way ANOVA with repeated measurements over time. Fisher’s post hoc test was used for comparison of group means relative to a control mean. The level of significance required for all post hoc analyses was p < 0.05. One-way ANOVA with repeated measures was used for comparison of drug values to a baseline mean. Two-way ANOVA was performed to determine the influence of 8-OH-DPAT on EMD- and fluoxetine-elicited increases in extracellular 5-HT. The new baseline value (100%) was defined by the mean of the two samples following EMD or fluoxetine injection. The values were determined from the first sample following 8-OH-DPAT administration to the end of sample collection.

Behavioral Testing. To determine whether EMD 68843 displayed antidepressant-like activity similar to that observed for fluoxetine and other SSRIs, we subjected rats to the FST, a test frequently used to reliably screen for effective antidepressants. Sprague-Dawley rats (Charles River Laboratories Inc., Wilmington, MA; 175–200 g at the start of the experiment) were housed in groups of two in polycarbonate cages and maintained on a 12:12-h light/dark cycle in a temperature-controlled (22 °C) colony with free access to food and water. Animals were handled for 5 min daily, beginning 4 days prior to the onset of the experiment. Animals were randomly assigned to four groups (n = 7–8 rats/group) and were exposed to a pretest swim session for 15 min 24 h prior to the 5-min swim test. Rats were placed into a glass cylinder (46 cm high × 20 cm wide) to a 30-cm depth with water at room temperature for 15 min. Animals were removed from the water, dried by the experimenter, and placed into plastic cages with a heating pad. EMD 68843 (1.0, 3.0, and 10.0 mg/kg) or vehicle (6% DMSO) was administered s.c. three times following the initial 15-min pretest exposure to the swim chamber. Injections were given 23.5, 4.5, and 1 h prior to the swim test. The 5-min swim session was videotaped from above for subsequent analysis of behavioral responses by a trained observer (Detke et al., 1995a). The rater was blind with respect to treatment group. Swimming was defined as horizontal movement through the swim chamber, which included crossing into another quadrant. Swimming activity consisted of upward-directed movements of the forepaws along the side of the swim chamber, and immobility was assigned when no activity was observed other than that necessary to keep the rat’s head above the water.

Further support for an antidepressant action of EMD 68843 was obtained by testing this compound in another species. The DBA/2J strain of mouse has recently been shown to be responsive to 5-HT obtained by testing this compound in another species. The DBA/2J mouse was purchased from The Jackson Laboratory, Bar Harbor, ME; 5 strain of mouse has recently been shown to be responsive to 5-HT. Prior to the swim session, and immobility was assigned when no activity was observed during the session. The 5-min swim session was videotaped from above for subsequent analysis. The last 4 min of the swim session were scored for behavior, which was defined as either immobile or active. The total amount of time spent immobile was the dependent measure used for statistical analysis.

5-HT Behavioral Syndrome. Rats were observed for 15 min for the appearance of the 5-HT behavioral syndrome immediately after the administration of 8-OH-DPAT (1.0 mg/kg, i.p.) or EMD 68843 (3.0 mg/kg, i.p.). Varying doses of EMD 68843 (0.3–3.0 mg/kg i.p.) were also administered 15 min prior to a challenge dose of 8-OH-DPAT (1.0 mg/kg, i.p.) to determine whether EMD 68843 could block the 5-HT syndrome. The rats were observed in individual polycarbonate cages with the floor covered with bedding material. The cage was similar to their home cage. Rats were rated for the occurrence of the following symptoms following drug injection: 1) flat body posture; 2) hindlimb abduction; 3) lateral head weaving; 4) resting tremor; 5) forepaw treading; and 6) Straub tail. Each symptom was rated on an intensity scale with 0 = absent, 1 = weak, 2 = moderate, and 3 = intense. An intensity score of 2 or greater was required for the symptom to be considered as prominently presented. Rats were scored as showing the 5-HT behavioral syndrome if at least four of the six symptoms were prominently presented during the observation period. These procedures have previously been used by this laboratory to rate the 5-HT behavioral syndrome produced by full agonists (Lucki, 1992; Singh and Lucki, 1993).

5[35]S[1]GTPγS Binding. Ligand-promoted binding of [35]S[1]GTPγS to G proteins with the 5-HT1A receptor expressed in Sf9 cells was used to compare the relative efficacy of compounds and was evaluated as previously described (Butkerait et al., 1995; Barr and Manning, 1997). 8-OH-PIPAT defines the effects of a full agonist, and vehicle (or DMSO) defines receptor constitutive activity. In the present study, [35]S[1]GTPγS binding achieved with 8-OH-PIPAT was compared with that achieved with EMD 68843, fluoxetine, and vehicle.

Drugs. All drugs were prepared freshly on the day of use. EMD 68843 (Merck, Darmstadt, Germany) was dissolved in 6% DMSO and injected i.p. in a volume of 2 ml/kg in the rat studies. Fluoxetine hydrochloride (Lilly Research Laboratories, Indianapolis, IN) was dissolved in deionized water and injected i.p. in a volume of 2 ml/kg. 8-OH-DPAT hydrobromide (Sigma/RBI, Natick, MA) was dissolved in deionized water. Fluoxetine and 8-OH-DPAT doses were calculated as the base weight. EMD 68843 doses were calculated without correction. Drugs were administered in a volume of 10 ml/kg in the mouse studies.

Results

Microdialysis Studies

Extracellular 5-HT Levels in the Ventral Hippocampus. Administration of fluoxetine (10 mg/kg) increased extracellular levels of 5-HT to a maximum of 192.2 ± 37.2% above basal values in the HPv. A higher dose of fluoxetine (20 mg/kg) increased extracellular 5-HT by 273.9 ± 77.1%. Two-way ANOVA revealed a significant increase in extracellular 5-HT in the HPv at both doses but no significant difference between dose [F(1,13) = 0.99, N.S.; time: F(9,117) = 10.52, p < 0.0001; dose × time interaction: F(9,117) = 1.00, N.S.; see Fig. 1A]. In contrast, EMD 68843 dose-dependently increased extracellular 5-HT in the HPv [F(4,26) = 14.8, p < 0.0001; time: F(9,234) = 20.75, p < 0.0001; dose × time interaction: F(36,234) = 3.36, p < 0.0001; see Fig. 1B]. The largest increase was observed following the 3 mg/kg dose of EMD 68843 (558.3 ± 52.5% above baseline).

Extracellular 5-HT Levels in the Frontal Cortex. Both doses of fluoxetine resulted in significantly elevated levels of extracellular 5-HT compared with saline injection as revealed by two-way ANOVA [F(2,16) = 15.07, p < 0.0002; time: F(9,144) = 7.02, p < 0.0001; dose × time interaction: F(18,144) = 1.87, p = 0.01; see Fig. 2A]. The peak
The ability of the 5-HT1A receptor agonist, 8-OH-DPAT, to increase extracellular levels of 5-HT in the ventral hippocampus (HPv). In contrast, although the 1 mg/kg dose elicited a maximum increase of 527.0 ± 28.3% above baseline, there was no significant difference between the two doses. Panel B shows the effect of several doses of EMD 68843: 0.3 mg/kg [squares (n = 6); baseline = 7.1 ± 1.3 fmol/10 μl]; 1.0 mg/kg [diamonds (n = 7); baseline = 5.9 ± 1.1 fmol/10 μl]; 3.0 mg/kg [triangles (n = 6); baseline = 6.3 ± 1.4 fmol/10 μl]; and 10.0 mg/kg [circles (n = 6); baseline = 4.7 ± 0.7 fmol/10 μl]. Post hoc analysis showed a significant dose-related increase in extracellular 5-HT levels elicited by all doses of EMD 68843, except 0.3 mg/kg (Fisher’s PLSD, p < 0.05).

Fig. 1. The effects of fluoxetine (panel A) and EMD 68843 (panel B) on extracellular 5-HT levels in the ventral hippocampus. Panel A shows that both the 10 mg/kg [squares (n = 7); baseline = 4.2 ± 0.8 fmol/10 μl] and 20 mg/kg [circles (n = 8); baseline = 6.9 ± 1.3] dose of FLX increased extracellular levels of 5-HT in the ventral hippocampus. However, there was no significant difference between the two doses. Panel B shows the effect of several doses of EMD 68843: 0.3 mg/kg [squares (n = 6); baseline = 7.1 ± 1.3 fmol/10 μl]; 1.0 mg/kg [diamonds (n = 7); baseline = 5.9 ± 1.1 fmol/10 μl]; 3.0 mg/kg [triangles (n = 6); baseline = 6.3 ± 1.4 fmol/10 μl]; and 10.0 mg/kg [circles (n = 6); baseline = 4.7 ± 0.7 fmol/10 μl]. Post hoc analysis showed a significant dose-related increase in extracellular 5-HT levels elicited by all doses of EMD 68843, except 0.3 mg/kg (Fisher’s PLSD, p < 0.05).

Fig. 2. The effects of fluoxetine (panel A) and EMD 68843 (panel B) on extracellular 5-HT levels in the frontal cortex. Panel A illustrates the significant increase of extracellular 5-HT in the frontal cortex with either fluoxetine or EMD 68843 was examined in the FC and the HPv. In the HPv, 8-OH-DPAT reduced extracellular 5-HT to the same extent in fluoxetine- and EMD-treated animals [drug: F(1,12) = 1.66, N.S.; time: F(6,72) = 7.55, p < 0.0001; drug × time interaction: F(6,72) = 0.89, p < N.S., two-way ANOVA; see Fig. 3A]. In contrast, 8-OH-DPAT reduced extracellular 5-HT in the FC of fluoxetine-treated animals to near basal levels, whereas only a brief reduction of 5-HT was noted in the EMD-treated animals [drug: F(1,12) = 2.90, N.S.; time: F(6,72) = 3.31, p = 0.006; drug × time interaction: F(6, 72) = 2.84, p = 0.016; see Fig. 3B].

Behavioral Studies

**Forced Swimming Test in Rats.** EMD 68843 (1.0 mg/kg) significantly reduced the amount of time spent immobile [F(3,27) = 3.67, p < 0.03]. A concomitant increase was evident in the amount of time spent swimming [F(3,27) = 4.68, p < 0.01], but no significant change was noted in climbing.
Follow-up tests confirmed that only the 1.0 mg/kg dose produced significant changes of immobility and swimming when compared with vehicle (see Fig. 4B).

Forced Swimming Test in Mice. EMD 68843 displayed antidepressant-like activity in the mouse FST at the 1.0 mg/kg dose. A significant decrease in immobility was revealed by one-way ANOVA \([F(3,27) = 3.36, p < 0.03]\). No significant changes were observed with the 0.3 or 3.0 mg/kg dose (see Fig. 4A).

Evaluation of Efficacy at 5-HT\(_{1A}\) Receptors

Sf9 Cells. Ligand-facilitated binding of \(^{35}\text{S}\)GTP\(_{\gamma}\)S to Gi proteins together with the 5-HT\(_{1A}\) receptor expressed in Sf9 cells was used to evaluate relative efficacy (Butkerait et al., 1995; Barr and Manning, 1997). As shown in Fig. 5, 5-HT ligands differentially increased \(^{35}\text{S}\)GTP\(_{\gamma}\)S binding \([F(4,10) = 249.9, p < 0.001]\). The full agonist 8-OH-PIPAT increased \(^{35}\text{S}\)GTP\(_{\gamma}\)S binding about 6-fold over that observed with vehicle \((p < 0.001)\). EMD 68843 increased binding by about 4-fold,
5-HT1A receptor agonist 8-OH-DPAT produced all of the symptoms of the 5-HT behavioral syndrome. In contrast, EMD 68843 when tested alone at the highest dose used (3.0 mg/kg, i.p.) produced none of the symptoms. Pretreatment with EMD 68843 dose dependently blocked the ability of 8-OH-DPAT to evoke the 5-HT behavioral syndrome at doses of 1.0 and 3.0 mg/kg (see Table 1). Pretreatment with fluoxetine did not alter the 5-HT syndrome produced by 8-OH-DPAT.

5-HT Syndrome in Rats. The administration of the 5-HT1A receptor agonist 8-OH-DPAT produced all of the symptoms of the 5-HT behavioral syndrome. In contrast, EMD 68843 when tested alone at the highest dose used (3.0 mg/kg, i.p.) produced none of the symptoms. Pretreatment with EMD 68843 dose dependently blocked the ability of 8-OH-DPAT to evoke the 5-HT behavioral syndrome at doses of 1.0 and 3.0 mg/kg (see Table 1). Pretreatment with fluoxetine did not alter the 5-HT syndrome produced by 8-OH-DPAT.

Discussion

As a ligand that potently combines blockade of 5-HT reuptake with high affinity for 5-HT1A receptors, the partial agonist EMD 68843 may be a prototype for a more effective type of SSRI. The additional regulation of 5-HT1A autoreceptors by a 5-HT transporter inhibitor may allow a single molecule to evoke responses that are similar to those of drug combinations that could augment the clinical effects of conventional SSRIs in treating depression, such as the combination of fluoxetine with pindolol. This hypothesis was tested using in vivo microdialysis to show that systemic administration of EMD 68843 increased extracellular levels of 5-HT in the FC (527 versus 165%) and the HPv (558 versus 274%) of the rat to a greater extent than the conventional SSRI fluoxetine. Although the two compounds differ in potency at 5-HT transporters, the effects produced by systemic fluoxetine are representative maximal effects for SSRIs, as shown for other more potent SSRIs (reviewed by Fuller, 1994; Hjorth et al., 2000) or for fluoxetine tested at higher doses (up to 40 mg/kg, i.p.; Lucki, unpublished data). This finding provides an important proof of principle for the conceptual development of novel compounds with dual actions on the 5-HT transporter and 5-HT1A receptors that may elicit a faster onset or more therapeutically effective antidepressant response.

The augmentation of extracellular 5-HT achieved by acute administration of EMD 68843 is likely due to the combined inhibition of the 5-HT transporter with partial agonist effects at the 5-HT1A autoreceptor. SSRI and 5-HT1A Ligand Augments 5-HT Neurotransmission 1225

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Table 1
EMD 68843 prevents the serotonin syndrome induced by 8-OH-DPAT

Rats were observed for 15 min after drug administration and scored for the intensity, appearance, and degree of expression of the six behavioral components of the serotonin syndrome on a 0 to 3 scale. Values shown are mean intensity scores on 0 to 3 scale for each symptom. Rats that displayed a score of 2 or higher on four or more signs were considered to display the serotonin syndrome (n = 6 rats for each group).
5-HT levels (Fletcher et al., 1993; Romero et al., 1996; Gartside et al., 1999; Hjorth et al., 2000). However, the mechanism underlying this effect is not easily understood because 5-HT1A receptor partial agonists produce complex effects. Partial agonists activate presynaptic 5-HT1A receptors but block the effects of 5-HT1A receptor agonists at postsynaptic receptors because of the different receptor reserve of 5-HT1A receptors at these anatomical locations (Cox et al., 1993). Although 5-HT1A receptor partial agonists inhibit 5-HT neuronal activity and reduce 5-HT synthesis, 5-HT1A autoreceptor activation by a full agonist, such as the endogenous neurotransmitter, can be diminished by pretreatment with a partial agonist (Arborelius et al., 2000). Nevertheless, additional pharmacological mechanisms may contribute to augmenting extracellular 5-HT levels in terminal regions when 5-HT1A receptor partial agonists are combined with SSRIs. One possible mechanism may be that partial agonists rapidly desensitize 5-HT1A autoreceptors, thereby inhibiting their functional activity and reducing their ability to inhibit 5-HT transmission (Romero et al., 1996). Other possible mechanisms could involve postsynaptic 5-HT1A receptors in terminal regions that participate in an inhibitory feedback loop to negatively regulate 5-HT transmission (Bosker et al., 1997; Casanovas et al., 1999) or direct effects on 5-HT release.

An important determinant of the effects of fluoxetine and EMD 68843 was the brain region being sampled. Previous microdialysis studies have described differential regulation of 5-HT transmission by the 5-HT1A autoreceptor in distinct brain regions (Kreiss and Lucki, 1994, 1997; Malagie et al., 1996; Invernizzi et al., 1997; Romero and Artigas, 1997; Knobelmann et al., 2001). Extracellular 5-HT levels demonstrate regional differences in sensitivity to 5-HT1A autoreceptor regulation, as shown previously when the 5-HT1A receptor antagonist WAY 100635 potentiated the effects of fluoxetine to a greater extent in the FC than in the dorsal hippocampus (Hervas and Artigas, 1998). Preferential innervation of the FC by serotonergic fibers from the DR and the innervation of the HPv by MR fibers (Kosofsky and Molliver, 1987) and greater responsiveness of DR neurons to the autoinhibitory actions of 5-HT on 5-HT1A receptors (Sinton and Fallon, 1988; Blier et al., 1990; Romero and Artigas, 1997) may contribute to regional differences in the response to SSRIs. Also, fluoxetine appeared to induce a smaller increase of extracellular 5-HT in the frontal cortex than in the HPv, which is comparable with findings from other laboratories (Fuller et al., 1994; Kreiss and Lucki, 1995; Invernizzi et al., 1996; Hervas and Artigas, 1998).

EMD 68843 also elicited regional changes in extracellular 5-HT. The greater increase in the HPv than the FC with the low (0.3 mg/kg) dose reflected regional differences in the response to systemic administration of SSRIs. The 3.0 mg/kg dose increased extracellular 5-HT levels in the FC for a longer time than the HPv, possibly reflecting the influence of blockade of 5-HT1A receptors in a region under greater control by the 5-HT1A autoreceptor.

The involvement of 5-HT1A autoreceptor mechanisms was addressed by examining the ability of the 5-HT1A receptor agonist, 8-OH-DPAT, to reduce the elevated 5-HT levels achieved by EMD 68843 or fluoxetine administration. In the FC, 8-OH-DPAT reduced extracellular 5-HT levels to near baseline levels in fluoxetine-treated animals only, whereas the EMD 68843-treated animals demonstrated only a transient reduction of extracellular 5-HT in response to 8-OH-DPAT. This result is consistent with EMD 68843 interfering with the 5-HT1A autoreceptor to limit the actions of 8-OH-DPAT as an autoreceptor agonist. Thus, the augmented levels of extracellular 5-HT produced by 3 mg/kg EMD 68843 probably involve the combination of 5-HT reuptake inhibition and interference with 5-HT1A autoreceptors regulating 5-HT transmission in the FC. In contrast, in the HPv, fluoxetine- or EMD 68843-induced increases in extracellular 5-HT levels were attenuated in a similar fashion by subsequent systemic administration of 8-OH-DPAT, suggesting that 5-HT1A autoreceptor function may exert less regulation of extracellular 5-HT and the effects of EMD 68843 in this region.

EMD 68843 was examined for its ability to produce the 5-HT syndrome and its interaction with the full 5-HT1A receptor agonist 8-OH-DPAT. The 5-HT behavioral syndrome is a series of unconditioned behavioral responses in rats evoked to study the activation of postsynaptic 5-HT1A receptors in vivo (Lucki, 1992). The behavioral syndrome is also an animal model for a serious side effect produced in humans by a number of antidepressant drugs (Gillman, 1998). EMD 68843 did not produce the 5-HT syndrome and completely prevented the actions of the more efficacious 5-HT1A receptor agonist 8-OH-DPAT on the 5-HT behavioral syndrome. These results are similar to effects shown previously by other 5-HT1A receptor partial agonists and antagonists (Singh and Lucki, 1993; Detke et al., 1995b) because only high-efficacy agonists can produce the 5-HT behavioral syndrome. Although a recent finding suggested that the full 5-HT1A agonist flibanserin might block certain components of the 5-HT syndrome induced by 8-OH-DPAT (Borsini et al., 2001), this interference was attributed to the likely involvement of flibanserin with 5-HT2A receptors. This is not the case for EMD 68843.

The behavioral results in the mouse and rat FST provide some support for the antidepressant potential of EMD 68843 but over a limited dose range. In the rat FST, EMD 68843 reduced immobility and increased swimming behavior. This response pattern is similar to that shown many times to be produced by conventional SSRIs, such as fluoxetine (10–20 mg/kg, s.c.), and is mediated by 5-HT (Lucki, 1997; Page et al., 1999). In the mouse FST, EMD 68843 reduced immobility in DBA/2J mice similar to fluoxetine (5–20 mg/kg, i.p.; Lucki et al., 2001). These findings are consistent with the idea that antidepressant-like actions are mediated through the activation of postsynaptic 5-HT receptors. However, doses of EMD 68843 higher than 1 mg/kg were ineffective in both the mouse and the rat FST. Although multiple 5-HT receptors may be involved in antidepressant behavioral responses, activation of postsynaptic 5-HT1A receptors appears to be important for producing behavioral effects of SSRIs in antidepressant tests (Lucki et al., 1994; De Vry, 1995). Pretreatment with 5-HT1A receptor partial agonists or antagonists, or genetic deletion of 5-HT1A receptors can prevent the behavioral effects of SSRIs (Singh and Lucki, 1993; Redrobe and Bourin, 1998; Mayorga et al., 2001; Reneric et al., 2001). Thus, although the 5-HT1A receptor partial agonist component of EMD 68843 may enhance extracellular 5-HT levels, at high doses it could also restrain expression of antidepressant-like behaviors in the FST, which predict clinical activity.

These findings have direct clinical implications for the development of faster-acting antidepressant compounds. The greater elevation of extracellular 5-HT levels achieved by EMD 68843 compared with fluoxetine can improve SSRI response and is qualitatively similar to augmentation effects achieved by the combination of SSRIs with pindolol (Perez, 1997; McAskill et al., 1998; Olver et al., 2000). A single molecule producing more efficacious
enhancement of extracellular 5-HT may also be more advantageous for other indications of SSRIs and circumvent potential pharmacokinetic interactions caused by the use of drug combinations (Olver et al., 2000). However, preclinical studies implicate the activation of postsynaptic 5-HT1A receptors as important for antidepressant responses to SSRIs (see Lucki et al., 1994; Blier et al., 1997; Cryan and Leonard, 2000). Whether the 5-HT1A receptor partial agonist component of EMD6843 would impede or facilitate antidepressant responses when tested clinically is unknown. In conclusion, the present findings demonstrate that a single compound with dual effects on 5-HT transporters and 5-HT1A receptors can produce a greater impact on extracellular 5-HT levels than conventional SSRIs. These intrinsically augmented effects may contribute to increasing the therapeutic effects of serotonergic antidepressants.

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References


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